IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Robert David Possee, *et al.*	)	
	)	Examiner: **Marvich, Maria**
Serial No. **09/807,809**	)	
(National Phase of PCT/GB00/03114	)	Art Unit: **1636**
	)	
Filed: **July 30, 2001**	)	
	)	
For: **BACULOVIRUS EXPRESSION SYSTEM**	)	

DECLARATION OF ROBERT DAVID POSSEE, PH.D UNDER 37 C.F.R. § 1.32

I, Robert David Possee, currently residing at 64 Millwood End, Long Hanborough, Witney, Oxon, OX29 8BY, UK, do hereby declare:

1. I am an expert in the field of the invention. I am currently a researcher at NERC CEH Oxford (formerly Institute of Virology and Environmental Microbiology/Institute of Virology). I earned a B.Sc. degree in Biological Sciences with Honours in 1978 at the University of Birmingham, UK. I earned a Ph.D. degree in virology in 1981 at University of Warwick, UK. My *curriculum vitae* is enclosed (Exhibit A). I published over one hundred papers and books in the field of biology. The list of the publications is enclosed (Exhibit B). I am co-author, with Linda King, who is a named inventor of the US Patent Application Serial No. 09/807,809, of a text book on baculovirus expression systems entitled "*The Baculovirus Expression System; a Laboratory Guide*" (1992), first ed., Chapman and Hall Publishers, London, UK (hereinafter referred to as "my text book"), which was submitted to the US Patent and Trademark Office with the Information Disclosure Statement filed April 18, 2003. I am a named inventor of US Patent Application 09/807,809 (hereinafter referred to as "the present application"), entitled "Baculovirus Expression System". I am familiar with the application and the Office Action mailed by the United States Patent and Trademark Office on 10 September 2007.

2. I am familiar with US 6,911,206 (Campos). Campos does not provide any evidence that it uses a circular, replication deficient baculovirus vector capable of being maintained in a bacterial cell.

3. One of ordinary skill in the art would understand that Campos does not provide any information about the actual baculovirus vector used. The experimental evidence provided in Campos is not reproducible without that information.

4. The Examiner alleges that Kool (PNAS (1994), **91**: 11212-11216) shows that Campos uses a circular baculovirus vector. It does not. Kool relates to the original wild-type baculovirus itself. It does not relate to baculovirus vectors which are the result of extensive modification of the original baculovirus genome. One of ordinary skill in the art would understand that the evidence Campos provides is that a linear vector is used.

5. The only information provided by Campos about specific vectors are about transfer vectors (pBak 8 & 9). These are used to make pBacHISgD:LH. One of ordinary skill in the art would understand that these are usually used with a complementary vector. pBak 8 & 9 are made by Clontech. I attach, as an Annex, a copy of a printout from the Clontech Website showing that the complementary expression vector is BacPAK6. This is sold as a Bsu36I digest. This is a restriction enzyme cut vector. That is, it is a linear (not circular) vector. The Examiner will also note that I am author on the two references referred to on that page.

6. The methods in Campos use linearised vectors. The absence of a step showing linearisation confirms that the vector was supplied as a linear vector. Linear DNA cannot be used in a one step protocol to produce recombinant virus. This is in contrast to the currently claimed method.

7. One of ordinary skill in the art would understand that evidence provided in Campos is that the vector used is not replication deficient. Example 4 of Campos states that "Repeated cycles of Sf21 cell infection and plaque assay purification can be performed to obtain a greater concentration". This is only required if the vector is NOT replication deficient. One of ordinary skill would understand that plaque purification is required to reduce the amount of non-recombined (parent) vector not having the gene of interest, but still being able to replicate. The current invention is replication deficient and does not require the plaque assay.

8. One of ordinary skill in the art would understand that using linearised baculovirus DNA may recircularise without the insertion of the foreign target DNA. This means that contaminant parental baculovirus is present. The evidence presented in Campos shows that parental DNA is being produced in the host cells. This necessitates plaque selection to avoid parental DNA not containing the target gene being grown on. The use of circular baculovirus avoids this problem. This is not suggested in the prior art documents of record.

9. I have not been able to identify any evidence to show that the baculovector used in Campos is capable of being maintained in a bacterial cell. There is no information provided in Campos about the structure of the vector, so the ability to be maintained in a bacterial host is not demonstrated. One of ordinary skill in the art would understand that baculovirus vector would need to be provided with bacterial nucleic acid sequences such as bacterial origins of replication to allow the vector to replicate in bacteria. There is no evidence of this. In the absence of this information one of ordinary skill in the art would understand that the baculovirus vector must be assumed to be maintained in an insect cell, but not a bacterial cell.

10. There is no evidence to show what sequences are provided to restore replication of the vector (as alleged by Campos). One of ordinary skill in the art would have difficulty reproducing the experiments alleged to have been carried out without this information.

11. In conclusion, one of ordinary skill in the art would understand that the evidence provided in Campos is that a linear, replication enabled baculovirus vector not capable of being maintained in bacteria was used.

12. One of ordinary skill in the art would understand that the claimed invention is not obvious in the light of Campos or Merrington (*Virology* (1996), pages 338-348).

13. One of ordinary skill in the art would understand that Campos does not disclose a circular, replication enabled, baculovirus not capable of being maintained in a bacterial cell. This is discussed in detail above. The advantages of the claimed method have been previously discussed in my Declaration dated 17 January 2005. To summarise, these are:

I. The claimed method allows for production of a pure population of recombinant baculovirus not contaminated with the parental baculovirus, or the baculovirus without insertions of foreign DNA. This considerably reduces the number of passages through cells that are generally required to remove the parental contamination. The effect of having to spend time to pick recombinant stocks to remove this contamination is disclosed in the application, for example, in the table on page 4, for conventional techniques used in linear DNA. Thus, the present invention avoids parental virus contamination and saves considerable time and money in the laboratory.

II. The use of the intermediate host allows the production of large quantities of baculovirus DNA, which has the defective virus gene and so cannot replicate alone when transfected into insect cells. It only replicates in insect cells if a transfer vector with a foreign gene and a functioning copy of the defective gene is transfected into the insect cell so as to restore the baculovirus genome. It is not possible to produce this defective baculovirus DNA in insect cells because of the defect in the baculovirus genome.

III. The intermediate host allows the baculovirus to be easily maintained and to be amplified to give large amounts of virus DNA.

IV. Using naked purified viral DNA that is not within viral particles allows the material to be stably stored, for example, in a refrigerator. Viral stocks contained within viral capsids suffer from problems of low infectivity when stored. In my laboratory, we have naked baculovirus DNA that is still stable after ten years of storage.

V. Using naked DNA allows the baculovirus vector and foreign DNA to be co-transfected, rather than having a two-step system where the virus is infected, and then foreign DNA is transfected in a separate step. This saves time and money.

VI. The separate introduction of virus particles and plasmid DNA of previous methods is not considered by those of ordinary skill in the art to be an efficient process for the production of recombinants. DNA introduced by a viral particle will

begin replication. Unless the incorporation of the virus particles and the plasmid DNA is timed correctly, there can be zero production of recombinant viruses. The claimed method overcomes this problem.

14. Prior art techniques utilise linearised baculoviral DNA. This is evidenced, for example, by the Annex under "BD BacPAK method". The linearised BacPAK baculovirus vector recombines with a rescue vector containing a target gene. One of ordinary skill would understand that this is not the method currently claimed. A copy of the article by Kitts & Possee is already of record. This describes BacPAK6. I am an author of that paper and confirm that it shows that BacPAK6 has a median of 95% of non-parental plaques which are recombinant. One skilled in the art would understand that this is much lower than 100% efficiency of the present invention. One skilled in the art would also understand that the paper also shows that the prior art technique of using linear DNA, such as BacPAK6 produces lower target gene expression than the current invention.

15. The Examiner has referred to Merrington. I confirm that one of ordinary skill would understand that lef-2 is not demonstrated as being inactivated. The paper merely shows a reduction in efficiency of viral replication. See page 343 lines 19-21. The paper does not show that lef-2 can be used to rescue a replication deficient virus. The abstract (penultimate line) and Figure 6 show that lef-2 did not have an effect on DNA replication.

16. One of ordinary skill in the art would not combine the teaching of Campos with Merrington or be motivated to derive the invention from the publications of record.

17. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of any patent issuing on this application.

RDPosse

Robert David Possee, Ph.D.

23/10/2007

Date

CURRICULUM VITAE

NAME: Robert David Possee

DATE OF BIRTH: 6th September 1956

BIRTHPLACE: Canterbury, England

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EDUCATION:

1968-1975	Secondary Education	9 O levels, 3 A levels, 1 S level
1975-1978	University of Birmingham	B.Sc. (Hons) 2.i, Biological Sciences
1978-1981	University of Warwick	Ph.D., Virology

Supervisor Prof. N.J. Dimmock
PhD thesis:"The Mechanism of the Neutralization of Influenza Viruses by Antibody".

PROFESSIONAL CAREER:

1981-present, NERC CEH Oxford (formerly Institute of Virology and Environmental Microbiology/Institute of Virology).

1981-1984, appointed Higher Scientific Officer (HSO) in Dr David Kelly's group as part of a project funded by NRDC to investigate the use of cell culture to produce baculoviruses for insect pest control. My specific duties were to develop molecular methods for the analysis of baculoviruses and novel insect cell lines. This laid the foundation for later work on virus expression vectors, recombinant protein production in cell culture and work on other baculoviruses such as *Panolis flammea* and *Mamestra brassicae* NPVs.

1984-1986, appointed Project Leader (HSO) of Baculovirus research group after departure of David Kelly. Initiated work on baculovirus expression vector system, which was disseminated within the Institute and remains a staple science at Oxford to the present day.

1986-1990, promoted to Senior Scientific Officer (SSO). Continued work on baculovirus expression vectors. Highlights include elucidation of the polyhedrin and p10 gene promoter structure and construction of expression vectors appropriate for the production of polyhedrin positive viruses and production of more than one foreign protein. This technology was key to the later development of recombinant baculovirus insecticides containing genes encoding insecticidal products.

1990-1999, promoted Unified Grade 7 (Principal Scientific Officer). A period that saw the construction and field testing of a recombinant baculovirus containing a gene encoding an insect-specific scorpion toxin gene. The completion of the first baculovirus genome sequence laid the foundation for future studies on genome function. An improved method for producing recombinant baculoviruses was developed and licensed to three companies. In 1992, I co-authored the first book detailing the necessary technologies required to undertake baculovirus expression vector work. After years of speculation, it was also discovered that insects can

harbour baculoviruses as persistent infections (at least in the laboratory, but see below for further work), challenging the dogma that virus occlusion bodies are the sole means of survival in the environment. The mechanism whereby baculoviruses liquefy their hosts was also revealed to involve at least two gene products: chitinase and cathepsin.
In 1993 I undertook a period of sabbatical leave in the laboratory of Professor Lois K. Miller in Athens, Georgia, USA. This provided a valuable insight to how one of the most successful scientists in the field operated and gave me the opportunity to develop some research ideas in the laboratory. I was also appointed visiting professor at Oxford Brookes University in 1998.

1999-present, Individual Merit Promotion (Band 3). This award has given me the freedom to pursue further novel lines of research, which in the face of a declining science budget, would have been difficult to attempt. Of particular current interest is the dissection of heterogeneous baculovirus populations by whole genome cloning in bacteria. This has revealed extensive virus population diversity in natural isolates, without the need for further amplification in insect hosts in the laboratory, which may introduce artifacts. A spin-off from this approach has been the development of an improved method for making recombinant baculovirus expression vectors, which was patented. I am currently developing DNA Microarray methods for analysing baculovirus gene expression. A collaborative project on baculovirus latency with Dr Rosie Hails and Prof. Linda King has demonstrated that wild populations of insects may also harbour persistent infections and offer an alternative way for the virus to survive in the field when host numbers are low.

2002-2003, appointed Deputy Director, CEH Oxford

2003-2004, Acting Director, CEH Oxford

2004 – present, Head of Department, CEH Oxford

SUPERVISION/MANAGEMENT OF STAFF:

Since 1984, I have supervised 4 science budget supported core research staff, 20 post-doctoral research fellows, 19 graduate students (PhD/D.Phil) and 4 research assistants. I have also hosted 7 visiting scientists from overseas. I have also had other management responsibilities involving the workshop staff at CEH Oxford and a photographer.

MANAGEMENT TRAINING:

Attended weeklong residential JTS course on Development for Senior Managers in 1998 and a half day course on communicating with the media. More recently, attended a one-day training course on safety for senior staff in Swindon.

TEACHING EXPERIENCE:

I have taught on undergraduate courses at Oxford University and Oxford Brookes University. I have also served as an external examiner for the University of London, University of Southampton, and as an internal examiner for the University of Oxford. PhD theses have also been examined from France and India.
Other extramural teaching has involved assisting the running of practical workshops on baculovirus expression vectors in Oxford, Paris, Argentina and Brazil

SCIENTIFIC MEETINGS ORGANISED:

I co-organised two International Workshops on Baculovirus Expression Vectors, in Oxford in 1988 and 1990. I also co-organised the Baculovirus workshop at the International Congress of Virology, Glasgow, 1993. I was a member of the Scientific Advisory Committee for Baculovirus and Insect Cell Gene Expression Conferences at Pinehurst, NC USA in 1995 and in Jersey in 1997. Large DNA

virus workshops have also been organized at various meetings of the Society for General Microbiology in the UK.

EDITORIAL BOARDS/INTERNATIONAL COMMITTEES:

I have been a member of the Virology Editorial Board since 1988; similarly for Journal of General Virology 1989 - 1992 and the Insect Virus Editor for the same journal since 1995. I was a member of the ICTV Baculovirus study group, 1990-1996 and am currently a scientific advisor to the EU Animal Cell Technology Industrial Platform (ACTIP).

CURRENT MEMBERSHIPS OF PROFESSIONAL SOCIETIES:

American Society for Virology
Society for General Microbiology
Society for Invertebrate Pathology
American Society for Microbiology
Biochemical Society
European Society for Animal Cell Technology UK

GRANTS/CONTRACTS AWARDED:

1. NERC Special Topic Award (1986-1989)
"Risk Assessment of the Release into the Environment of Genetically Engineered Baculovirus Insecticides". £60,000 (1 HSO/PDF)

2. NERC CASE Studentship (1986-1989)
CASE award with Professor R. Southwood, Dept of Zoology, Oxford University
"Molecular Biology of *Autographa californica* nuclear polyhedrosis virus"

3. Department of the Environment (1986-1989)
"The Risk Assessment of Genetically Engineered Baculovirus Insecticides"
£200,000 (1HSO/PDF, 1 SO/technician) + equipment (ABI DNA sequencer, £86,000).

4. NERC CASE Studentship (1987-1990)
CASE Award with Dr L.A. King, Oxford Brookes University
"Biological and Genetic Diversity of Baculoviruses"

5. Wellcome Environmental Health (1988-1991)
"The development of genetically engineered baculoviruses as novel, safe, specific
and environmentally acceptable insecticides" £200,000 (2 HSO/PDFs).

6. EC Biotechnology Action Programme (1989-1991)
"Risk Assessment of the Field Use of Genetically Engineered Baculoviruses"
£45,000 (1SSO/PDF). In collaboration with Dr J. Vlak, Wageningen, Netherlands.

7. NERC CASE Studentship (1989-1992)
CASE award with Dr L.A. King, Oxford Brookes University
"Latent baculovirus infections in field and laboratory insect populations"

8. NATO Collaborative research grant - for travel (1990-1994)
"Development of genetically engineered baculovirus insecticides containing modified JHE genes"£5,000.
In collaboration with Dr S. Maeda and Prof. B. Hammock, Davis, California.

9. Department of the Environment (1990-1993)
"Risk assessment of baculovirus insecticides" £379,000 (3 HSO/PDF).

10. NERC CASE Studentship (1991-1994)
NERC CASE award with Dr L.A. King Oxford Brookes University
"Analysis of baculovirus late gene expression factors"

11. Pfizer (1991-1994)
DPhil studentship
"Baculovirus expression vectors" £53,000.

12. Glaxo (1991-1994)
DPhil studentship
"Production of recombinant proteins using baculoviruses and insect cells" £50,000.
13. EC BRIDGE Programme (1992-1994)
"Biosafety of genetically modified baculoviruses" £85,000 (1HSO/PDF)
In collaboration with Dr J. Vlak and Dr J. Huber, Darmstadt, Germany.
14. NERC CASE Studentship (1992-1995)
CASE award with Dr L.A. King, Oxford Brookes University
"Baculovirus molecular biology"
Student resigned studentship in first year; award terminated
15. EC BIOTECH Programme (1993-1996)
"Baculovirus RNA polymerases" £135,000 (1 HSO)
In collaboration with: Dr D Knebel-Moersdorf, Cologne, Germany; Dr M. Lopez-Ferber, San Christol, France.
16. NERC CASE Studentship (1993-1996)
CASE award with Dr L.A. King, Oxford Brookes University
"The role of chitinase in the baculovirus infection in insects"
17. NERC Faraday Studentships (1993-1996)
2 awards with Dr L.A. King, Oxford Brookes University and Dr J. Windass (Zeneca Agrochemicals) "Genetic engineering of baculovirus insecticides"
2 awards with Professor D.H.L. Bishop, IVEM, Dr A. Kingsman, Oxford University and Dr T. French, British Biotechnology Ltd.
"Baculovirus expression vectors"
18. EC BIOTECH Programme (1994-1996)
"Minireplicon baculovirus expression vectors" £102,000 (1HSO)
In collaboration with Dr Just Vlak, Wageningen, Netherlands; Dr Dagmar Knebel-Moersdorf, Cologne, Germany; Dr Miguel Lopez-Ferber, San Christol, France; Dr Christian Oker-Blom, Turku, Finland.
19. Pfizer (1994 - 1997)
PhD studentship "Baculovirus expression vectors" £50,000 (PhD)
20. NERC CASE Studentship (1995-1998)
CASE award with Dr L.A. King, Oxford Brookes University
"Genetic and biological analysis of host range determinants in baculoviruses".
21. NERC Small Research Grant (1996-1997)
"Analysis of field collected insects for the presence of persistant baculovirus infections" £22,777.
With Dr L.A. King, Oxford Brookes University
22. NERC Standard Research Grant (1996-1998)
"Evolutionary and biological significance of baculovirus chitinases"
With Dr L.A. King, Oxford Brookes University
23. BBSRC Standard Research Grant (1996-1999)
"Baculovirus RNA polymerases" £138,000
With Dr L.A. King, Oxford Brookes University
24. NERC EDGE Programme Grant (1998-2001)
"Pathogen variability and dynamics in insect populations" £305,167
With Drs J.S. Cory and R.S. Hails (IVEM and Drs A.D. Watt and S. Hartley (ITE Banchory).
25. NERC EDGE Programme Grant (1998-2001)
"Genetic variation and the dynamics of pathogens in host-pathogen interactions" (£250,000 with Dr J.S. Cory (IVEM); Prof. M Begon and Dr D.J. Thompson (Liverpool).
26. EC BIOTECH FP IV (1998-2000)
"Baculovirus surface display - Developments and applications" £372,400 total (£108,500 [UK award] with Dr I.M. Jones (IVEM); Dr C. Oker-Blom (University of Helsinki); Dr R. Grabherr (Institute of Applied Microbiology, Vienna; Dr J. McCafferty (Cambridge Antibody Technology Ltd.
27. HSE (2001 - 2003)

"Development of an air sampling/biosensor system to detect genetically modified viruses" £35,000.

28. CEH Integrating fund (2001 - 2004)

"Interactions between viruses and lepidopteran larvae in different stress states" £150,000. Jointly held with Dr Daniel Osborn, CEH Monks Wood.

29. NERC CEH New blood position (2001 – 2004)

"Bioinformatics" £150,000. Jointly with Prof. Mark Bailey.

30. NERC Innovation fund (2000 – 2002)

"Development of baculovirus expression vectors" £52,337

31. NERC non-thematic (2001 - 2004)

"The ecological and evolutionary significance of latent baculovirus infections in insects". £282,846. Jointly held with Dr Rosie Hails (CEH), Prof. Linda King (Oxford Brookes University) and Dr Steve Sait (University of Liverpool).

32. OST Molecular Infrastructure Award (2001)

£750,000. Submitted on behalf of CEH and in collaboration with Prof. Mark Bailey (CEH Oxford).

PUBLICATIONS

1. **Possee, R. D. and Dimmock, N. J. (1981).** Neutralization of influenzavirus by antibody: attachment, uptake and uncoating of neutralized virus in chick embryo cells. In: *Genetic Variation Among Influenza Viruses*, pp. 473-480. ICN-UCLA Symposium XXI. Edited by D. P. Nayak & C. F. Fox. London: Academic Press.
2. **Possee, R.D. and Dimmock, N.J. (1981).** studies on the mechanism of neutralization of influenza virus by antibody: Evidence that neutralizing antibody (anti-haemagglutinin) inactivates influenza virus *in vivo* by inhibiting virion transcriptase activity. *Journal of General Virology* **58**, 373-386.
3. **Possee, R. D. (1986).** Cell-surface expression of influenza virus haemagglutinin in insect cells using a baculovirus vector. *Virus Research* **5**, 43-47.
4. **Howard, S. C., Ayres, M. D. and Possee, R. D. (1986).** Mapping the 5' and 3' ends of *Autographa californica* polyhedrin mRNA. *Virus Research* **5**, 109-119.
5. **Matsuura, Y. Possee, R. D. and Bishop, D. H. L. (1986).** Expression of the S-coded genes of lymphocytic choriomeningitis arena virus using a baculovirus vector. *Journal of General Virology* **67**, 1515-1529.
6. **Moore, N. F., King, L. A. and Possee, R. D. (1986).** Viruses of Insects. *Insect Science Applications* **8**, 275-289.
7. **Matsuura, Y., Possee, R. D. Overton, H. A. and Bishop, D. H. L. (1987).** Baculovirus expression vectors: the requirements for high level expression of proteins, including glycoproteins. *Journal of General Virology* **68**, 1233-1250.
8. **Possee R. D. and Howard, S. C. (1987).** Analysis of the polyhedrin gene promoter of the *Autographa californica* nuclear polyhedrosis virus. *Nucleic Acids Research* **15**, 10233-10248.
9. **Possee R.D., Cameron, I.R., Allen, C.J. and Bishop, D.H.L. (1988).** Experiences with the first field release of genetically engineered viruses. In: *Viren und Plasmide in der Umwelt.* Schriftenreihe des Vereins fur Wasser-, Boden- und Lufthygiene **78**, p165-186. Eds. J.M. Lopez Pila, E. Seber and K. Jander. Gustav Fischer Verlag, Stuttgart/NewYork.
10. **Possee, R. D. and Kelly, D. C. (1988).** Physical maps and comparative DNA hybridization of *Mamestra brassicae* and *Panolis flammea* nuclear polyhedrosis virus genomes. *Journal of General Virology* **69**, 1285-1298.
11. **Bishop, D.H.L., Entwistle, P.F., Cameron, I.R., Allen, C.J. and Possee, R.D. (1988).** Field trials of genetically-engineered baculovirus insecticides. In: *The Release of Genetically-Engineered Micro-organisms* Eds. M. Sussman, C.H. Collins, F.A. Skinner and D.E. Stewart-Tull. Academic Press London.
12. **Weyer, U. and Possee, R.D. (1988).** Functional analysis of the p10 gene 5' leader sequence of the *Autographa californica* nuclear polyhedrosis virus. *Nucleic Acids Research* **16**, 3635-3653.
13. **Bishop, D.H.L., Entwistle, P.F., Cameron, I.R., Allen, C.J. and Possee, R.D. (1988).** Genetically engineered baculovirus insecticides. *Aspects of Applied Biology* **17**, 385-395.
14. **Weyer, U. and Possee, R.D. (1989).** Analysis of the promoter of the *Autographa californica* nuclear polyhedrosis virus p10 gene. *Journal of General Virology* **70**, 203-208.
15. **Cameron, I.R. and Possee, R.D. (1989).** Conservation of polyhedrin gene promoter function between *Autographa californica* and *Mamestra brassicae* nuclear polyhedrosis viruses. *Virus Research* **12**, 183-199.
16. **Oakey, R., Cameron. I.R., Davis, B., Davis, E. and Possee, R.D. (1989).** Nucleotide sequence and transcription mapping of the polyhedrin gene of the *Panolis flammea* nuclear polyhedrosis virus . *Journal of General Virology* **70**, 769-775.
17. **Possee, R.D. and Bishop, D.H.L. (1989).** Areas of uncertainty in the uncontained use of modified organisms in the environment: The viruses from the molecular viewpoint. Proceedings of the European Biosafety Workshop: The Uncontained Use of Modified Organisms in the Environment.
18. **Cameron, I.R., Possee, R.D. and Bishop, D.H.L. (1989).** Insect cell culture technology in baculovirus expression systems. *Trends in Biotechnology* **7**, 66-70.
19. **Bishop, D.H.L. and Possee, R.D. (1990).** Baculovirus expression vectors. *Advances in Gene Technology* **1**, 55-90.
20. **Possee, R.D., Allen, C.J., Entwistle, P.F. Cameron, I.R. and Bishop, D.H.L. (1990).** Field trials of genetically engineered baculovirus insecticides. In: *Risk Assessment in Agricultural Biotechnology*, Proceedings of the International Conference, (J.J. Marois and G. Bruening, eds.) University of California.

61. **Possee, R.D., Cory, J.S., Hirst, M. and Bishop, D.H.L. (1993).** Field tests with genetically engineered baculoviruses. In: *BCPC monologue no. 55, Opportunities for molecular biology in crop protection.* (Eds. D.J. Beadle, D.H.L. Bishop, L.G. Copping, G.K. Dixon, D.W. Holloman).

62. **King, L.A., Possee, R.D., Atkinson, A., Palmer, C., Marlow, S., Pickering, J. and Beadle, D. (1994).** Advances in Insect Virology. In: *Advances in Insect Physiology* 25, 1-73. Academic Press. London.

63. **Possee, R.D. and King, L.A. (1994).** Molecular approaches to the design of biotic crop protection agents. (1994). In: *Molecular Perspectives in Crop Protection* (Eds. G. Marshall and D. R. Walters) pp 68-97. Chapman and Hall.

64. **King, L.A., Mann, S.G., Lawrie, A.M. and Possee, R.D. (1994).** Production and isolation of recombinant baculoviruses. In: *Cell Biology Handbook* (Ed. K. Celis) Academic Press, New York. pp. 148-154.

65. **Ayres, M.D., Howard, S.C., Kuzio, J., Lopez-Ferber, M. and Possee, R.D. (1994).** The complete DNA sequence of *Autographa californica* nuclear polyhedrosis virus. *Virology* 202, 586-605.

66. **Hughes, D.S., Possee, R.D. and King, L.A. (1994).** Quantitation of latent *Mamestra brassicae* nuclear polyhedrosis virus DNA. *Journal of Virological Methods* 50, 21-28.

67. **Bonning, B.C., Roelvinck, P.W., Vlak, J.M., Possee, R.D. and Hammock, B.D. (1994).** Superior expression of juvenile hormone esterase and beta-galactosidase from the basic protein gene promoter of *Autographa californica* nuclear polyhedrosis virus compared to the p10 and polyhedrin gene promoters. *Journal of General Virology* 75, 1551-1556.

68. **Cory, J.S., Hirst, M.L., Williams, T., Hails ,R.S., Goulson, D., Green, B.M., Carty,T.M., Possee, R.D., Cayley, P.J. and Bishop, D.H.L. (1994).** Field trial of a genetically improved baculovirus insecticide. *Nature* 370, 138-140.

69. **Possee, R.D. (1995).** Risk assessment and field trial with a genetically modified baculovirus insecticide. Proceedings from: *Pan-European conference on the potential long-term ecological impact of genetically modified organisms.* pp.147-163. Council of Europe Press.

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BOOK

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BD BacPAK™ Baculovirus Expression System

- *Express proteins at high levels—1 to 500 mg of protein per liter of culture*
- *Retain the biological activity of expressed proteins*
- *High recombinant efficiency*
- *Vectors compatible with BD Creator™ technology are available*

The BD BacPAK™ Baculovirus Expression System expresses recombinant proteins at extremely high levels in insect host cells (1, 2). The BD BacPAK™ System offers three major advantages:

- **High yield of recombinant protein.** The insect host cells produce large amounts of your target protein.
- **Greater similarity to naturally occurring proteins.** The expressed recombinant protein is usually similar in structure, biological activity, and immunological reactivity to the naturally occurring protein because insect host cells provide post-translational processing similar to that of mammalian cells.
- **High recombination efficiency.** More than 90% of the viruses produced by the transfected cells carry the target protein. The specially designed BD BacPAK6™ Viral DNA forces recombination between the virus and transfer vector, resulting in high recombination efficiency.

BD BacPAK™ method

The target gene is inserted into a shuttle vector, which is cotransfected into insect host cells with the linearized BD BacPAK6 Viral DNA. The BD BacPAK6 DNA is missing an essential portion of the baculovirus genome. When the DNA recombines with the vector, the essential element is restored and the target gene is transferred to the baculovirus genome. Following recombination, a few viral plaques are picked and purified, and the recombinant phenotype is verified. The newly isolated recombinant virus can then be amplified and used to infect insect cell cultures to produce large amounts of the desired protein.

The BD BacPAK System includes the transfer vectors, BD BacPAK6 Viral DNA, the insect host cells needed for production of recombinant proteins and BD™ Bacfectin Transfection Reagent for high efficiency transfections. A User Manual, sequencing/PCR primers, and control viruses are also included with the kit. For rapid determination of baculovirus titers, we recommend the BD BacPAK™ Rapid Titer Kit (Cat. No. 631406). BD BacPAK6 Viral DNA can also be purchased separately.

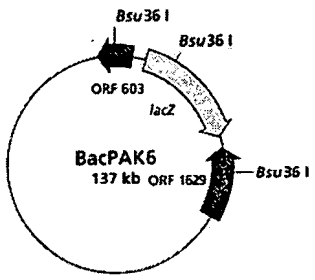

Figure 1. BD BacPAK6™ Viral DNA map.

Ordering Information

Product	Size	Cat. No.
BD BacPAK Baculovirus Expression System	each	631402
BD BacPAK6 DNA (*Bsu*36 I digest)	5 transfxns	631401
pLP-BacPAK9 Acceptor Vector	20 µg	631407
pLP-BacPAK9-6xHN Acceptor Vector	20 µg	631408

Components

pBacPAK8 Transfer Vector
pBacPAK9 Transfer Vector
BD BacPAK6 Viral DNA (*Bsu*36 I digest)
BD Bacfectin Transfection Reagent
IPLB-Sf21 *Spodoptera frugiperda* Cells
BD BacPAK6 Virus Stock (positive control)
AcMNPV Wild-Type Virus (negative control)
Bac1 Sequencing/PCR Primer
Bac2 Sequencing/PCR Primer
pBacPAK8-GUS Positive Control Transfer Vector
User Manual (PT1260-1)
Protocol-at-a-Glance (PT1260-2)

Storage Conditions

–180°C for IPLB-Sf21 Insect Host Cells
4°C for all other components

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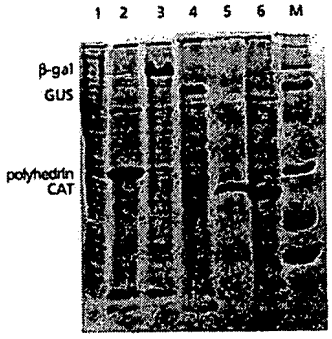

Figure 2. Protein production from recombinant viruses generated using the BD BacPAK™ Baculovirus Expression System. Recombinant viruses were obtained by cotransfection of transfer vectors with BD BacPAK6 Viral DNA (*Bsu*36 I digest), followed by amplification in Sf21 cells. The SDS PAGE analysis of cellular lysates was performed 48 hr after infection of the Sf21 cultures. Lane 1: uninfected Sf21 cells. Lane 2: Sf21 cells infected with wild-type AcMNPV virus. Lane 3: Sf21 cells infected with nonrecombinant BacPAK6 virus. Lane 4: Sf21 cells infected with BacPAK8-GUS recombinant virus. Lane 5: purified CAT protein. Lane 6: Sf21 cells infected with BacPAK9-CAT recombinant virus. Lane M: molecular weight marker.